

Production of Egg Yolk Coloring Material by a Fermentation Process¹

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ABSTRACT

SCHWARZ, Y. (Technion—Israel Institute of Technology, Haifa, Israel), AND P. MARGALITH. Production of egg yolk coloring material by a fermentation process. Appl. Microbiol. **13**:876–881. 1965.—*Rhodotorula mucilaginosa* was cultivated in a yeast propagator, for the production of biomass and carotenoids. A spray-dried preparation of the yeast was incorporated into the diets of laying chickens for the promotion of egg yolk color. Several aspects of the effect of media on growth and carotenogenesis of pigmented yeast were examined. Addition of 1 to 2% of *Rhodotorula* yeast to the feeding mixture considerably improved egg yolk color. The possibility of using carotenogenic yeasts as a feed supplement is suggested.

Although carotenogenesis in microbial systems has been studied by various authors (*see reviews by* Haxo, 1955; Goodwin, 1963), the fermentative production of carotenoids, most of all β -carotene, has been realized on a pilot-plant scale with only a few organisms, chiefly of hyphal character, such as members of the Choanephoraceae and Mucoraceae (Anderson et al., 1958, Ciegler, Arnold, and Anderson, 1959a, b). Most of the work has been done with *Blakeslea trispora*, with a view of making the process economically feasible. Good yields of β -carotene have been obtained by use of cheap raw materials from natural sources, chemical steering factors (e.g. α - or β -ionone, a nonionic detergent), and specific mating types (Ciegler, Nelson, and Hall, 1962, 1963). Several attempts to employ other organisms, such as pigmented yeasts (Nakayama, Mackinney, and Phaff, 1954; Deufel and Clark, 1958), have so far met with little success, since only comparatively small amounts of β -carotene could be recovered under laboratory conditions.

The coloring capacity of chicken feed is of primary importance in modern poultry industry. Chickens raised in a farmer's yard usually get enough fresh plant material rich in xanthophylls to provide good coloring material to the egg yolk and often also to the skin or the shanks of broilers. However, in the poultry industry, where chickens are raised and fed in batteries, the problem of coloring material has not yet been solved

for various reasons. First, it is not practical to supply fresh plant material in sufficient quantities for a large-scale poultry industry. Second, preparations of xanthophyll-rich plant material, such as dried alfalfa meal, are of variable quality and low storage stability. Third, it is necessary to supply large amounts of such plant material, thus increasing the cellulose content of the diet to undesirable levels.

The demand for eggs with desirable yolk pigmentation is not universal, but many poultry industries exporting large quantities of these products are confronted with this problem. Although yolk color is practically without nutritive value, many importing countries insist on intense yolk pigmentation for the supply of their markets. In other cases, the supply for the meat industry has also met with difficulties due to the demand for better skin and shank coloration.

The coloring material of egg yolk consists chiefly of xanthophylls (lutein, zeaxanthin) and only small amounts of carotenes (Romanoff and Romanoff, 1949). According to Bourne (1960), the ratio is about 10:1 to 30:1. It has been further shown that chickens accumulate xanthophylls preferentially from a xanthophyll-carotene mixture, whereas carotenes alone are quite unsuitable for pigmentation purposes.

There have been several attempts to find substitutes for the expensive and unstable coloring material from phanerogam plant material. Dried algal meal from *Spongiococum excentricum* has been suggested (Morehouse, 1961). Synthetic xanthophylls, such as β -apo 8'-carotenal and β -apo 8'-carotenoic acid methyl ester, have been

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advocated by various authors (Steinegger and Zanetti, 1959; Rauch, 1959, 1960). These compounds are incorporated into the diet at a concentration of about 5 g per ton of feeding mixture, with good results. However, these compounds are still comparatively expensive, and, considering the current attitude of the public against the incorporation of organics into feedstuffs, there is still considerable interest in other coloring material from natural sources.

The purpose of this work was to evaluate the possibility of employing pigmented yeast material for poultry feeding, both as a source of vitamin concentrate and egg yolk pigmentation.

MATERIALS AND METHODS

Organism. *Rhodotorula mucilaginosa*, strain R33 from the Technion Culture Collection, was employed throughout this work. The organism has been previously identified according to Lodder and Kreger-van Rij (1952).

Materials. Media were prepared from ingredients from commercial sources. For large-scale fermentation work, chemically pure as well as technical-grade materials were used. β -Ionone was obtained from The British Drug House, Poole, England, and the nonionic detergents (Tweens) were from the Atlas Powder Co., Wilmington, Del.

Media. Cultures were maintained at 30 C on agar slants containing 0.2% yeast extract (Difco), 0.5% peptone (Difco), 2% glucose, and 2% agar, in distilled water at pH 6.8 to 7.0; cultures were transferred at weekly intervals. Propagations were carried out by use of yeast extract-peptone-glucose broth and chemically defined media containing a source of carbon, ammonium sulfate or ammonium lactate, minerals, and thiamine chloride. The composition of each medium is given below. Industrial materials such as molasses, soya bean meal, corn steep liquor, etc., were examined as fermentation raw materials in the pilot plant. Media were autoclaved for 20 min at 121 C. Glucose was sterilized separately and added aseptically to the medium.

Propagation. The yeasts were grown aseptically in shaker flasks or nonaseptically in a yeast propagator, consisting of a stainless-steel vessel with a perforated bottom, through which air was dispersed without agitation or baffles. A working volume of 20 liters was employed. At this stage of work, the propagation of *Rhodotorula* was carried out chiefly under nonaseptic conditions because of economic reasons which will be referred to in the Discussion.

Analysis. For routine purposes, the total carotenoid content of a yeast culture was determined as follows. Cells from 75 ml of the culture broth were harvested by centrifugation ($2,500 \times g$ for 20 min). The precipitate was washed once with cold water and transferred to a stainless-steel Waring Blendor. After blending for 1 min five times, the broken cells were centrifuged and hydrolyzed for

30 min in 30 ml of 0.5 N HCl in a boiling-water bath.

The cells were cooled in ice water for 10 min and recovered by centrifugation. The pigments were extracted with two portions of 30 ml of acetone, keeping the second portion in a cold room overnight before decantation. This procedure usually resulted in a complete extraction of the pigments, although sometimes an additional hydrolysis was necessary. The combined acetone fractions were extracted with two portions of 30 ml of hexane (boiling point, 68 to 70 C). The separation of layers was facilitated by the addition of small amounts of water. The combined hexane fractions were washed with 50 ml of water, dried over sodium sulfate, and brought to volume. The total carotenoid content was determined in a Klett-Summers colorimeter with a blue filter (400 to 465 m μ). A freshly prepared solution of potassium dichromate was used as a standard for each set of determinations. Values of total carotenoids, expressed as β -carotene, were computed from a standard curve of dichromate versus β -carotene (85% pure all *trans* β -carotene from Nutritional Biochemicals Corp., Cleveland, Ohio). This procedure was employed for an approximate evaluation of carotenogenesis, and should be considered only for comparative purposes.

The quantitative determination of the various carotenoid fractions synthesized by *Rhodotorula* was carried out according to Peterson *et al.* (1958), by use of a column chromatography procedure. Dry weight determinations were carried out on washed cells recovered from 10 ml of culture broth and dried overnight at 105 C. Sugars were determined according to Somogyi (1954), and the formol number was determined according to the standard method of the Association of Official Agricultural Chemists (1960).

The evaluation of egg yolk color was carried out during the field experiment by use of a color fan (Hoffmann-La Roche, Inc., Nutley, N.J.). By this method, the yolk color may be described from bright lemon (no. 1) to dark orange-red (no. 12). According to this scale, no. 7 is considered a satisfactory color for egg yolk, and no. 8-9 is a most desirable reddish-yellow pigmentation.

The determination of egg yolk color was made daily for four eggs randomly chosen from each group of birds.

Field experiments. The evaluation of the coloring material was carried out in a small poultry battery consisting of 8 groups of 12 birds each, of Leghorn \times Rock chicken. Details of the organization and nutritional aspects of these experiments will be reported elsewhere. Here, only the results of the feeding experiment with regard to carotenoid supply and the level of yolk color obtained will be recorded.

RESULTS

Carbon and nitrogen sources for the propagation of *R. mucilaginosa* were tested for their pro-

duction of dry matter and carotenogenesis. These experiments were carried out in 500-ml shaker flasks with 100 ml medium; 2 ml of a 48-hr culture in yeast extract-peptone-glucose medium served as inoculum. Cultures were incubated on a rotary shaker (200 rev/min) at 30 C for 6 days. Typical results of these experiments are summarized in Table 1.

The effect of pH on the growth of *R. mucilaginosa* was investigated to determine whether the yeast could be grown under nonaseptic conditions. At pH levels below 3.0, the propagation of the cells was considerably reduced. This was of special importance when ammonium sulfate was employed as a nitrogen source, resulting in very low pH values of the medium. Interestingly, carotenogenesis per unit of dry weight was little affected when growth was inhibited by low pH.

For similar reasons, we attempted to replace yeast extract with thiamine chloride for the growth of *R. mucilaginosa*. This proved successful at a level of 1 ppm; increased concentrations of thiamine up to 100 ppm were without effect on carotenogenesis.

In view of the results obtained with *B. trispora* and other carotenogenic fungi (Anderson et al.,

1958), several attempts were made to stimulate carotenoid production by the addition of various compounds, such as α and β ionone, as well as detergents, oils, etc. As shown in Table 2, non-ionic surface agents such as Tween 40 had a slight but significant effect on total carotenogenesis.

TABLE 2. Effect of various compounds on growth and carotenogenesis in *Rhodotorula mucilaginosa*

Compound added*	Dry wt	Total carotenogenesis (mg)	
		Per g of dry wt	Per 100 ml of broth
None	2.6	1.8	4.2
Tween 40, 0.1%	2.6	1.9	5.0
α -Ionone† + Tween 40, 0.1% each	1.0	1.5	1.5
β -Ionone† + Tween 40, 0.1% each	0.9	1.4	1.3
Tween 80, 0.1%	2.5	1.7	4.2
Kerosene, 2.0%, + Tween 40, 0.1%	1.6	1.4	2.3

* All additions were made to the ammonium lactate-glucose-minerals medium of Table 1.

† Added after 3 days of growth.

TABLE 1. Effect of some media on growth and carotenogenesis in *Rhodotorula mucilaginosa**

Carbon source	Other ingredients	Final pH	Dry wt	Total carotenoids (mg)	
				Per g of dry wt	Per 100 ml of broth
Glucose			%		
2%	Peptone, 0.5%; yeast extract, 0.2%	5.6	0.83	1.6	1.4
5%	(NH ₄) ₂ SO ₄ , 0.7%; yeast extract, 0.2%	2.0	0.98	1.4	1.4
10%	(NH ₄) ₂ SO ₄ , 1.5%; yeast extract, 0.2%	1.8	0.96	0.9	0.9
5%	(NH ₄) ₂ SO ₄ , 0.7%; yeast extract, 0.2%; K ₂ HPO ₄ , 0.5%; MgSO ₄ ·7H ₂ O, 0.05%	1.8	1.02	1.8	1.8
5%	(NH ₄) ₂ SO ₄ , 0.23%; urea, 0.11%; ammonium lactate, 0.38%; yeast extract and minerals as above	3.3	2.40	1.8	4.4
10%	(NH ₄) ₂ SO ₄ , 0.43%; urea, 0.22%; ammonium lactate, 0.75%; yeast extract and minerals as above	5.1	3.52	1.4	4.9
5%	Ammonium lactate, 1.15%; K ₂ HPO ₄ , 0.05%; MgSO ₄ ·7H ₂ O, 0.05%; thiamine chloride, 1 ppm	7.4	2.66	1.8	4.9
Beet molasses†					
50% sucrose					
10%	Thiamine chloride, 1 ppm	4.2	1.04	1.4	1.4
5%	Corn steep liquor, 50% solids, 5.0% yeast extract, 0.2%; K ₂ HPO ₄ , 0.05%; MgSO ₄ ·7H ₂ O, 0.05%	8.1	2.35	0.8	2.0

* All media were prepared with distilled water and adjusted to pH 6.6 to 6.8 before autoclaving. Ammonium lactate was prepared by neutralizing lactic acid with ammonium hydroxide to pH 6.0. For carotenoid analysis, see text.

† Beet molasses was treated with potassium ferrocyanide before use according to Gerhardt, Dorell, and Baldwin (1946).

genesis, whereas ionones inhibited both growth and carotenogenesis.

Pilot-plant propagation. *Rhodotorula* cells were grown in a 20-liter (working volume) yeast propagator aerated (2 volumes of air per volume per min) through a perforated-bottom disc, without agitation. Vessels were steam-treated for 30 min before introduction of media. Steam was then passed through coils for another 0.5 hr. After cooling, a 10% inoculum was introduced. No aseptic measures were employed during the fermentation run. Propagations were carried out at 26 C for 60 to 70 hr.

For production purposes, fermentations were run with glucose-lactate medium containing (grams per liter): glucose (anhydrous), 50.0; ammonium lactate, 11.5; K_2HPO_4 , 0.5; $MgSO_4 \cdot 7H_2O$, 0.5; Tween 80, 1.0; thiamine chloride, 1 ppm; pH, 5.2 to 5.5. Figure 1 describes a typical run. The use of glucose and ammonium lactate led to a rapid drop in pH, which was followed by the utilization of lactic acid and a later rise in pH. This behavior of hydrogen ion concentration seems to be ideal for the prevention of contamination during the main phases of growth. However, since it is possible that the growth of yeast in the presence of lactic acid may be suboptimal due to the inhibition of lactic acid at low pH values, the propagation of *Rhodotorula* was also attempted with ammonium sulfate as nitrogen source, adjusting the pH level to 5.0 with alkali. In this case, the rate of yeast growth and caro-

tenesis was considerably increased. The generation time in the glucose-lactate medium was about 15 hr, as compared with 5 hr in the glucose-ammonium sulfate medium with pH adjustment. However, in the absence of suitable equipment for the automatic control of pH, this procedure could not be adopted.

Preparation of dried yeast powder. Mature cultures from the propagators were harvested in a basket centrifuge equipped with a suitable filtering cloth. The red cells were resuspended in a small amount of water, and a thick suspension was fed to a Niro atomizer (Copenhagen) at a rate of 2 liters per hr. The entrance temperature was 160 C, and the exit temperature, 72 C. This operation yielded a powder with 8% moisture. About 5 kg of *Rhodotorula* cells were prepared in this way. Viability tests of the spray-dried yeast were negative. The powder was kept in a dark cold room until further use.

Feeding experiments. Eight groups of birds were fed ad libitum with diets as outlined in Table 3. To group 1, yellow corn and high quality alfalfa meal were added for standard yolk pigmentation (positive control). During the first week of the experiment, diets were devoid of carotenoids, which led to the depletion of coloring material from the birds' bodies. During the subsequent 14 days, diets were supplemented with various concentrations of yeast, except that of group 2 which served as a negative control. During the last week of the experiment, an acid-hydrolyzed

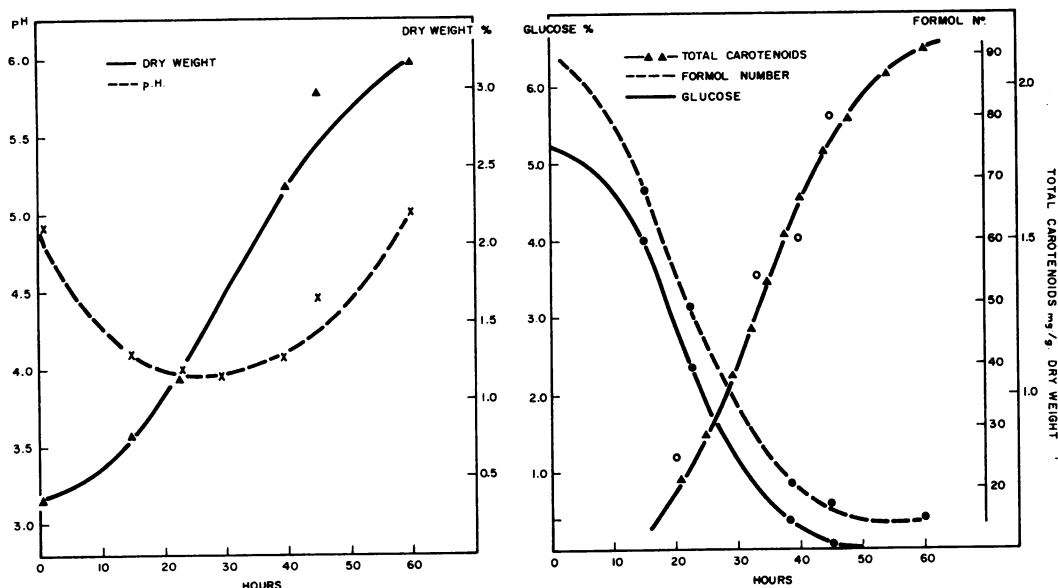


FIG. 1. Growth and carotenogenesis in *Rhodotorula mucilaginosa*. The medium contained (g/liter): glucose (anhydrous), 50.0; ammonium lactate, 11.5; K_2HPO_4 , 0.5; $MgSO_4 \cdot 7H_2O$, 0.5; Tween 80, 1.0; thiamine chloride, 1 ppm, pH 5.2 to 5.5.

TABLE 3. *Percentage composition of diets*

Component*	Group†							
	1	2	3	4	5	6	7	8
<i>Rhodotorula</i>	0	0	0.5	1.0	2.0	3.0	4.0	5.0
Yellow corn.....	64.5	0	0	0	0	0	0	0
Alfalfa meal.....	5	0	0	0	0	0	0	0
Sorghum.....	0	67.5	67.5	67.5	67.5	66.5	65.5	64.5
Soya bean meal (defatted).....	19.0	21.0	20.5	20.0	19.0	19.0	19.0	19.0

* In addition, diets contained: fish meal, 2%; chalk-gravel, 6.5%; dicalcium phosphate, 2.5%; a mineral mixture, 0.5%. A commercial vitamin preparation, Aureofac, and some basalt grains were also incorporated. After 21 days, groups 5, 6, 7, and 8 received 0.5, 0.5, 2, and 4% of a hydrolyzed yeast preparation, respectively.

† Each group consisted of 12 birds.

TABLE 4. *Effect of pigmented yeast on yolk color^a*

Group no.	Yeast in mixture	Color after 7 days of depletion	Color after 14 days of full mixture	Yeast hydrolysate added	Color 7 days after hydrolysate admixture	Total carotenoids ^b (mg/g of fresh yolk)
	%			%		
	Positive control ^c	7.8 ^d	8.3	—	8.7	26.1
2	Negative control	3.5	3.0	—	3.5	5.0
3	0.5	4.0	5.2	—	6.0	8.6
4	1.0	4.5	6.3	—	6.5	15.1
5	2	4.0	6.5	0.5	6.8	17.9
6	3	4.3	6.5	0.5	7.5	18.4
7	4	4.5	7.0	2	8.2	19.5
8	5	3.5	7.2	4	10.0 ^e	25.4

^a Color determination: average of four yolks.

^b Determined according to Peterson et al. (1958) on yolk at the end of the feeding experiment.

^c The positive control group received yellow corn and high-quality alfalfa meal; the negative control group got no carotenoid supplement.

^d Continuous feeding. No color depletion.

^e Unnatural, red pigmentation.

preparation of *Rhodotorula* cells was incorporated into the diets of half of the groups. The hydrolysate was prepared by suspending the cells in a 0.5 N HCl solution at a concentration of 10% (w/v) and boiling for 30 min. Thereafter, the suspension was neutralized, and the cells were recovered by decantation and filtration.

The carotenoid content of the feed yeast was determined on the hydrolyzed preparation. The following components were found ($\mu\text{g/g}$, dry weight): torularhodin, 90; torulene, 38; β -carotene, 7.

The results of the feeding experiment are described in Table 4. It is evident that *R. mucilaginosa* yeast affect appreciably the yolk pigmentation of chicken. A diet containing about 1 to 2% of dried yeast increased yolk color from 3.0 to 3.5 to 6.3 to 6.5. The incorporation of hydrolyzed yeast increased yolk color to even higher values,

although at higher doses undesirable red color was observed. A diet containing 2% of the hydrolyzed yeast preparation yielded egg yolk color similar to that of birds fed on 65% yellow corn and 5% dried high-quality alfalfa meal. Undoubtedly, hydrolysis of yeast cells made the carotenoids more available for the deposition in egg yolk.

DISCUSSION

Although the problem of egg yolk pigmentation is being solved commercially by the suitable incorporation of plant xanthophylls, such as yellow corn and alfalfa meal, or more recently also by the addition of synthetic coloring material of carotenoid nature, there is still widespread interest in efficient yolk color promoters of natural origin. This work was carried out to determine the yolk-coloring potential of pigmented yeast of the genus *Rhodotorula*, especially *R. mucilaginosa*.

Carotenogenic yeast propagated under pilot-plant conditions with the use of commercial nutrient ingredients were recovered and incorporated into chicken feed diets. These yeasts, and more particularly hydrolyzed preparations thereof, were found to affect considerably the yolk color in a feeding experiment carried out under field conditions.

The incorporation of food yeast into chicken diets is of wide commercial use. Yeasts are known to supply diets with high concentrations of vitamins of the B complex, the so-called "unidentified growth factors," protein, and minerals. The use of pigmented yeast might be of considerable interest, since it would also provide yolk-coloring material of natural origin without additional cellulose. The production of *Rhodotorula* yeasts, which in many aspects resembles food yeasts, with the advantage of a nonfermentative metabolism, should not meet with any outstanding difficulties. With suitable pH control, there should be no handicaps due to contamination even in a nonaseptic process, such as is employed for the production of baker's yeast (see White, 1954).

It is very likely that the use of pigmented yeast as such cannot at present be advocated for commercial use due to some very slight undesirable hue of the egg color. In spite of the significant improvement of yolk color due to yeast feeding, it has to be pointed out that the Hoffmann-La Roche color scale does not represent the whole aspect of yolk pigmentation as observed by the human eye. Egg yolk with a 8-9 color number showed a very slight violet background which may not be accepted by the public. However, it is very plausible that this difficulty can be overcome by the use of pigmented yeast as a coloring supplement, together with low concentrations of conventional xanthophyll-rich plant material. Further research on the biogenesis of yeast carotenoids should lead to the production of yeast preparations with a carotenoid composition which would impart more acceptable coloration to the egg yolk.

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LITERATURE CITED

- ANDERSON, R. F., M. ARNOLD, G. E. N. NELSON, AND A. CIEGLER. 1958. Microbiological production of beta-carotene in shaken flasks. *J. Agr. Food Chem.* **6**:543-545.
- ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS. 1960. *Methods of analysis*, 9th ed., p. 13.
- Association of Official Agricultural Chemists, Washington, D.C.
- BOURNE, G. H. 1960. World review on nutrition and dietetics, vol. 2, p. 135. Hafner Publishing Co., New York.
- CIEGLER, A., M. ARNOLD, AND R. F. ANDERSON. 1959a. Microbiological production of carotenoids. IV. Effect of various grains on production of beta-carotene by mated strains of *Blakeslea trispora*. *Appl. Microbiol.* **7**:94-98.
- CIEGLER, A., M. ARNOLD, AND R. F. ANDERSON. 1959b. Microbiological production of carotenoids. V. Effect of lipids and related substances on production of beta-carotene. *Appl. Microbiol.* **7**:98-101.
- CIEGLER, A., G. E. N. NELSON, AND H. H. HALL. 1962. Microbiological production of carotenoids. VIII. Influence of hydrocarbon on carotenogenesis by mated cultures of *Blakeslea trispora*. *Appl. Microbiol.* **10**:132-136.
- CIEGLER, A., G. E. N. NELSON, AND H. H. HALL. 1963. Enhancement of beta-carotene synthesis by citrus products. *Appl. Microbiol.* **11**:128-131.
- DEUFEL, R. D., AND F. M. CLARK. 1958. Production of beta-carotene by species in the genus *Rhodotorula*. *Bacteriol. Proc.*, p. 13.
- GERHARDT, P., W. V. DORELL, AND I. L. BALDWIN. 1946. Citric acid fermentation of beet molasses. *J. Bacteriol.* **52**:555-564.
- GOODWIN, T. W. 1963. The biosynthesis of vitamins and related compounds. Academic Press, Inc., New York.
- HAXO, F. T. 1955. Some biochemical aspects of fungal carotenoids. *Progr. Chem. Org. Nat. Prod.* **12**:169-197.
- LODDER, J., AND N. J. W. KREGER-VAN RIJ. 1952. The yeasts, a taxonomic study. North Holland Publishing Co., Amsterdam.
- MOREHOUSE, A. L. 1961. Dried algae meal as a source of xanthophyll for egg yolk pigmentation. *Poultry Sci.* **40**:1432.
- NAKAYAMA, T., G. MACKINNEY, AND H. J. PHAFF. 1954. Carotenoids in asporogenous yeasts. *Antonie van Leeuwenhoek J. Microbiol. Serol.* **20**:217-228.
- PETERSON, W., J. EVANS, E. LECCE, T. A. BELL, AND J. L. ETCHELLS. 1958. Quantitative determination of the carotenoids in yeasts of the genus *Rhodotorula*. *J. Bacteriol.* **75**:586-591.
- RAUCH, W. 1959. Über die Beeinflussung der Dotterfarbe durch carotinoidhaltiges Legehennenfutter. *Arch. Geflügelk.* **23**:319-331.
- RAUCH, W. 1960. Über die Beeinflussung der Dotterfarbe durch carotinoidhaltiges Legehennenfutter. *Arch. Geflügelk.* **24**:417-431.
- ROMANOFF, A. L., AND A. J. ROMANOFF. 1949. The avian egg. John Wiley & Sons, Inc., New York.
- SOMOGYI, M. 1954. A new reagent for the determination of sugars. *J. Biol. Chem.* **160**:61-68.
- STEINEGGER, P., AND G. ZANETTI. 1959. Versuch zur Ermittlung der Einflüsse verschiedener Carotinoidzusätze zum Legehennenfutter auf die Dotterfarbe. *Arch. Geflügelk.* **23**:166-173.
- WHITE, J. 1954. Yeast technology. John Wiley & Sons, Inc., New York.